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STUDIES ON REDUCTION IN PLANTS.[1]

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(WITH PLATES I–VI).

I. REDUCING DIVISION IN *ARISÆMA TRIPHYLLUM* BY RING- AND TETRAD-FORMATION DURING SPOROGENESIS.

IN connection with some studies carried on under my direction upon the embryology of certain of the Araceæ, an effort was made to investigate carefully the nuclear figures during the formation of the pollen from the mother cells of the archesporium. In *Arisæma triphyllum* the figures accompanying the heterotypic divisions are of such interest that it seems desirable to offer the results of the study as a contribution to our knowledge of this subject. Especially is this so because the evolution of the heterotypic figures is so divergent from that described for most of the plants heretofore studied; and in one phase coincides so plainly with certain of the types found in animals; while in another phase it departs, so far as I have yet been able to determine, from any type heretofore described.

Early in July the young leaves and the spadix of Arisæma, which are to appear the following spring, are formed, though quite small. They may be found by breaking the aerial shoot from the corm and removing several enveloping fleshy bud scales which protect the next season's leafy shoot. The stamens and pistils appear at this time as very minute protuberances on the

[1] Paper read before the Botanical Society of America, at the Boston meeting, August 1898.

surface of the spadix. During the latter part of July and early in August these become more prominent and begin to take on the character of stamens or pistils, as the case may be. The staminate plants can usually be determined during August and in September by the dried remnant of the staminate flower, which in many instances remains attached during the season; or by the cleft in the petiole of the leaf where it emerged. The pistillate plants can usually be determined by the presence of the forming fruit, though some of the smaller pistillate plants of one season become staminate for the next season, because of exhaustion in seed bearing. The archesporium of the staminate plants develops during July, August, and early September; or it may be mature by September.

The material for this study was collected and prepared by myself during September 1897. The staminate plants were brought to the laboratory and the material was fixed on the day of collection. The spadix was first removed from the bud so that the anthers were entirely exposed. One or two anthers from each spadix were removed and crushed on a glass slip in a drop of water for examination under the microscope to determine the stage of development. In this way all material where the pollen was already developed could be discarded, and only that preserved which was in one or another stage of mitosis. This made it possible to obtain good sections for study on a large number of the slides prepared. The nuclear figures can be quite readily seen in the living condition, but if there was any difficulty in determining the stage of mitosis, a drop of a solution of chloral hydrate was added, as suggested by Humphrey. The spores are formed centrifugally on the spadix, *i. e.*, the stamens at the base of the spadix are usually more advanced than those toward the apex, so that if the pollen was just formed in the lower stamens, the upper ones might show pollen mother cells in one or another of the stages of division. There was, however, considerable variation in the same stamen in this respect, while in a single locule there was little if any variation.

The material selected in this way was placed without further

preparation in Flemming's chrom-osmium-acetic solution for twenty-four hours. It was then washed in running water from twelve to twenty-four hours, dehydrated with grades of alcohol; decolorized by placing for twenty-four hours in bulk in hydrogen peroxid and alcohol, made by using 70 parts of a 95 per cent. alcohol and 30 parts of hydrogen peroxid. It was then stored in 75 per cent. alcohol after one rinsing. Six months later a portion of the material was placed in the hands of a student (Miss Susie P. Nichols) for study, and three months later the remainder was prepared on the slide for study by Mrs. W. A. Murrill under my direction. It was imbedded in paraffin, cut with a Minot-Zimmermann microtome 6.6–13.3 μ thick, stained, some in Flemming's triple safranin-gentian-violet-orange stain, and some with iron hæmatoxylin. For the present description of the material, and for the conclusions presented, the writer alone is responsible.

During the formation of the spirem the linin network gradually disappears, and a more or less continuous thread is formed. A distinct spirem was not observed, though this phase of mitosis has not yet been sufficiently studied. Even at the time of the longitudinal cleavage of the thread it was seen to anastomose more or less, where, in its windings, portions came in contact. At this time it presents the appearance of a very open, irregular network. The chromatin granules, at first distributed over the linin network, gradually accumulate in small masses scattered along the thread. As the spirem thread becomes more distinct it shortens, and accompanying this the small masses of chromatin increase in size, giving an appearance of a string of beads, or they present an irregular moniliform appearance. In addition to this beaded appearance of the thread the masses of chromatin are more or less irregular or angular. At some of the angles of the chromatin masses short delicate threads are attached, probably remnants of the linin reticulum which do not participate in the formation of the spirem. This, with the angularities of the chromatin masses, gives a somewhat ragged contour to the spirem. Quite early in the formation of the spirem, and before

the reticulum has completely disappeared, the thread shows a longitudinal splitting which is evident between the chromatin masses. In the early stages of the longitudinal splitting of the thread I have not seen the small chromatin masses divided. Each chromatin mass often appears to be made up of two smaller ones lying side by side, which have fused at the points of contact. Even in larger chromatin masses, and among the chromosomes in Arisæma, there is a strong tendency for those lying in contact to fuse more or less, so that the individuals of a single mass or of the chromosomes are often more or less obscured.

While the chromatin masses on the spirem are more or less fused, they are small and emarginate at opposite ends, and at a point coincident with the axis of the band. By this character one can distinguish the double nature, or paired condition of the masses. It suggests a division of the original masses, or the deposition of the chromatin in paired masses along the thread, which fuse more or less at the point of contact. These chromatin masses often stain unevenly, two portions separated by the axis of the thread or band taking on a deeper stain. The darker points lie in pairs along the thread, each separated by a paler chromatin area which runs for a short distance on either side along the division of the thread. At the point between the deeper staining masses the paler zones fuse.

As the spirem shortens, these paired chromatin masses are brought nearer together. Fusion among them takes place, so that the masses increase in length and breadth as the spirem broadens. The longitudinal division of the spirem is marked here and there, sometimes at quite regular intervals, by rounded, elliptical, or oblong openings in the chromatin, while in other places, for considerable distances, no line of division can be ascertained, though often the difference in the intensity of the stain marks the separation of the masses into pairs, while the paler surrounding zones are fused. As the band shortens, the contour becomes more irregular, showing crenations and irregularities, to which are often attached delicate strands, perhaps portions of the linin reticulum.

The chromosomes are marked off by the band separating into sixteen segments. So far as could be determined, this number is quite constant. The segments are not formed by an abrupt division of the spirem, but it appears as if the chromatin masses by shortening unite themselves into elongated areas, which are connected for a time by two delicate threads representing the original line of longitudinal division. The chromosomes lie around the periphery of the nuclear cavity and are quite variable in form; this is due to variations in the extent to which longitudinal fission has proceeded. In a few, longitudinal fission is complete, and a pair of long knotted rods is present. In others, there is a slight connection at one end or near the middle by the fusion of adjacent chromatin. In others, one or both ends of the flattened chromosome are indented or forked; in the latter case figures approaching the letters Y or X are formed. In many of the chromosomes the line of fission is shown by one or two small rounded openings or narrow open slits near the middle or near the ends; or the opening is long and extends from end to end, individuals of the pair being connected only at the end, thus resembling long irregular chain links. Some of the individuals of the pair lie parallel; others are separated more or less at the middle, when quite well defined rifts are formed. Still others resemble chain links twisted half way around, thus presenting a figure 8 appearance (*fig. 6*).

The length of the chromosomes is now from one third to one half or more the diameter of the nuclear cavity, and they show the irregularities presented by the spirem band, being knotted or angular along the edges or at the end. Delicate linin threads are often still attached at the knotted or angular enlargements.

The chromosomes now shorten, the chromatin becoming massed together, until their length is from one sixth to one fourth the diameter of the nuclear cavity. A larger proportion of them have now opened out in the middle in the form of rings, and, while they have shortened in length, their breadth is about the same as formerly, or they may be a little broader. This gives a more perfect ring form, but the chromosome is still somewhat longer than its

breadth, so that the line of longitudinal fission can be determined. The line of longitudinal fission is also indicated in other ways. Other chromosomes at this time are present in the form of short plates, some of which show slight indentations at the end, while in others longitudinal fission is complete, and a pair of rods is formed.

During the process of shortening of the chromosomes, the greater part of the chromatin usually becomes massed at four definite points in the ring, or plates, or pair of rods. These denser masses of chromatin lie near the ends of the rods, or near the ends of each lengthened half of the ring or plate, and are connected by paler staining areas, the density of which varies in different rings. These four masses of chromatin, in the ring or in the rods as the case may be, form the tetrad. These rings and tetrads in *Arisæma triphyllum* are very distinct, as much so as in certain animals. The two lengthened halves of the rings are usually more nearly separated along the line of longitudinal fission than along the line of transverse division, so that they quite readily separate. Each half of the ring is more or less crescent shaped, and the ends, instead of being rounded, are often quite regularly angular, presenting figures that frequently aid one in determining the orientation of the cleavage line. Up to this time the chromosomes still occupy their position around the periphery of the nuclear cavity, and, up to quite a late period, often show fragments of linin attached.

The nuclear membrane now disappears, and the kinoplasmic threads, which are to form the spindle, enter and move the chromosomes up to the nuclear plate. The threads first radiate irregularly in all directions, but converge more and more at two poles as they gradually form the spindle. As this takes place, there seem to be two centers of force which lie at the poles of the spindle, and occasionally this is manifested a short time before the spindle is complete by radiations of kinoplasm about the poles in the form of rays, which suggests a centrosphere figure (*fig. 14*). This was observed very plainly in one case, and perhaps similar figures in other plants have led observers to interpret them as centrospheres.

As the chromosomes are drawn to the nuclear plate they lie in various positions. At first it was thought that the axis of the chromosome, whether a ring, a pair of rods, or plate, lay perpendicular to the axis of the spindle. I have observed them in this position a short time before the spindle is complete. In some such cases, the position of the spindle threads suggests that the chromosomes are pulled around before division so that the axis is parallel with the spindle axis. In other preparations, the chromosomes, where they lie close together, tend to fuse to such an extent that it is impossible to determine in what position they lie at the nuclear plate, *i. e.*, whether parallel with or perpendicular to the axis. In a large number of preparations conditions are such as to lead me to believe that the axis of the chromosome is parallel with the axis of the spindle. The chromosomes are so numerous and of such size that they cannot all lie at the periphery of the plate, but occupy the center of the nuclear plate as well, so that an end view of the nuclear plate shows the chromosomes quite evenly distributed over this area. In many cases a large number of them are fused, and curious figures are thus presented. At other times they lie entirely separated, so that the individual rings, or pairs of rods, can be seen and counted in an end view of the nuclear plate. Where the section is cut so as to show an oblique view of the plate, the groups of tetrads, or pairs of rods, can be well seen and counted. From studies of sections in this direction, it appears that there are sixteen groups of tetrads, which would make sixty-four individual chromosomes, thirty-two to be distributed to each daughter nucleus of the first division, and perhaps sixteen to each daughter nucleus of the second division. A side view of the spindles of such preparations, at the nuclear plate stage, shows the long axis of the rings or pairs of rods to be parallel with the axis of the spindle. Since the chromosomes are distributed through the center of the nuclear plate as well, they lie like a bundle of chain links. The form of the ring, as suggested above, indicates that the axis of the longitudinal cleavage of the spirem or of the tetrad lies parallel with the axis of the spindle.

This is important in determining the character of the first division, as to whether it is to be a longitudinal or a transverse division of the chromosomes.

Not only does the form of the ring aid in determining the position of the chromosomes at the nuclear plate stage of the first division; the paired rods also serve to determine this. These lie parallel with the axis of the spindle. Even before the rings have moved to the nuclear plate there is a great tendency for them to separate into longitudinal halves. This occurs also at the nuclear plate stage. The tetrads separate more readily along the line of longitudinal division than they do on the line of transverse division. For this reason, at the nuclear plate stage, the figures with paired rods, rings partly or completely separated along the line of the lengthwise cleavage of the spirem, together with the form of the rings, should determine the position of the long axis of the chromosomes. The tufts of spindle fibers are attached to each individual of the tetrads, and transverse or reducing division of the chromosome results as these are drawn away from the nuclear plate. In many cases the sixty-four tetrads, thirty-two to each pole, move as distinct individuals, so that longitudinal, as well as transverse division, occurs in the first mitosis of the mother cell nucleus. But while transverse division occurs during the first mitosis of the nucleus, longitudinal division of the chromosome takes place first. The peculiarity of Arisæma, then, is that, while longitudinal division precedes transverse division of the chromosome, both divisions occur during the first or heterotypic division, and the real reduction follows soon the pseudo-reduction in the heterotypic division.

The only other account of a reducing division in the first mitosis which I have noticed is that by Korschelt in the study of the development of the egg of the annelid Orphryotrocha, quoted by Wilson in "The cell in development and inheritance," p. 201. During the first mitosis in oogenesis, the spirem segments into four chromosomes, which is the normal number in the somatic cells. These split, and then the pairs fuse

together again, the four chromosomes arranging themselves in a tetrad, two going to the first polar body and two remaining in the egg cell. These two split and form another tetrad, two chromosomes going to the second polar body and two remaining in the egg nucleus. This brings about reduction in the first mitosis.

Beliaieff[2] for a number of years has maintained that a reducing division takes place in plants, and, based on his studies of the division of the pollen mother cells of Iris, classifies the modes of division of the nucleus in plants into three types: (1) the vegetative division; (2) the heterotypic division; and (3) the reducing division. The heterotypic division takes place, as is well known, in the first mitosis of the pollen mother cell, and the reducing division accompanies the second mitosis.

Calkins[3] announces reducing division during the second mitosis of the pollen mother cell in Pteris and Adiantum. Calkins was unable to determine whether the reducing division in Pteris and Adiantum occurred during the primary or secondary mitosis, since the tetrads were packed so closely in the nuclear plate of the first division. During the secondary mitosis the diads elongate to form a rod. This elongation he thinks takes place in the direction of the axis of the spirem, and would thus show that the primary mitosis is an equation division, while reduction takes place during the secondary mitosis. Nevertheless he recognizes that it is immaterial whether reduction takes place during the first or second division. The formation of tetrads in the prophase of the primary mitosis is generally regarded as the preparation of the number of the chromosomes which are to be eventually distributed to the four daughter nuclei of the second mitosis; and where the tetrads are formed by one longitudinal and one transverse division of the segment of the spirem, it is equivalent to reduction in the prophase of the first division,

[2] BELIAIEFF, W.: Ueber die Reductionstheilung des Pflanzenkernes (Vorläufige Mittheilung). Ber. d. deutsch. bot. Gesells. **16**: 27–34. 1898.

[3] Chromatin reduction and tetrad formation in pteridophytes. Contrib. Dept. Bot. Columbia Univ. **5**: 101–115. *pl. 295, 296.* 1897.

though usually, so far as accounts go, the final separation for the reduction does not occur until the second division. In Arisæma the mode of tetrad formation by longitudinal and transverse division is such as to effect reduction in the prophase of the first division, while during metaphase and anaphase the reduction is completed by the separation of the tetrad of the transversely divided diad.

II. REDUCING DIVISION OF THE CHROMOSOMES IN *TRILLIUM GRANDIFLORUM* DURING SPOROGENESIS.

In studying the development of the pollen of *Trillium grandiflorum*, the chromosomes were found to be of such large size as to offer, possibly, a good opportunity for an investigation which might throw some light on certain of the problems of mitosis during sporogenesis. The preliminary studies were made upon material collected during the autumn and winter of 1896–7. With the experience of one season's work it was possible to plan for the collection of a quantity of material in different stages of development. The young flowers of Trillium begin their development in June and July of the previous year, and by autumn they are well formed, though they remain protected in the sheathing bud scales during the winter. So far as I have found, the archesporium is completed and the period of growth of the pollen mother cells is closed with the oncoming of winter, if not before. The period during which the division of the pollen mother cells takes place extends over seven or eight months. This is due not only to some variation in the time of maturing of different plants in different localities, but also to temperature. In the open woods, on rather high ground, where the plants are protected from cold north and west winds and are exposed to the warmth of the sun, the pollen is often mature in the latter part of September. In the cool ravines and open places exposed to north winds, the pollen is formed in warm days during February, March, or April, according to the season, and to some extent also according to the state of maturation in different individuals.

Very cold weather checks the progress of mitosis, but when

the mother cells have reached the proper state of maturation a few warm days in the winter or early spring months are sufficient for the two successive divisions. It appears that during the winter season, when the nights are cool and the days warm, the mitotic figures may be prolonged for several days. For this reason it is not difficult to prepare a large quantity of material in different stages of division. For the present study the material was collected during the month of February 1898. Some of this was growing in a garden where it was transplanted the year before, while the bulk of the material was collected from wooded ravines, from one to two miles distant from the laboratory, the plants being easily found by raking off the leaf covering on the ground.

All the material was examined before fixing in order to know just the stage of division in individual plants, and even assorted into lots showing the spirem stage, first and second division, for convenience in cutting material of any desired stage. During division the chromosomes are so large and the cytoplasm so clear, one can easily determine the different stages with a one sixth objective, so that desired material could be assorted into that showing prophase, nuclear plate, anaphase, etc. In each individual flower, the tip of one of the stamens was removed, crushed in a drop of water on the slide, and examined first with a two thirds objective, then with a one sixth. In this way undesirable material could be rejected.

Before placing in the Flemming solution, sometimes the tips of all the anthers were cut off, while other material was placed in the fixing solution with the anthers closed. After the removal of the sepals, petals, and the apex of the pistil, so that the fixing solution could enter the locules of the young carpels, the entire androecium still attached to the receptacle was thrown into the solution. This made it possible to section several anthers of an individual flower together.

The fixing solution used was Flemming's chrom-osmium-acetic solution. The material remained in this from fifteen to twenty-four hours, was washed from twelve to twenty hours in cold

running water, dehydrated in grades of alcohol to 95 per cent., then decolorized in mass in alcohol of 70 per cent. which was diluted with hydrogen peroxid. It was then passed back to alcohol of commercial strength, then through absolute alcohol, and cedar oil; finally it was infiltrated with paraffin and imbedded. It was then stored in the paraffin blocks for a few weeks or months as time became available for cutting and staining. The material was cut on a Minot-Zimmermann microtome and stained, some with the triple stain (safranin-gentian-violet-orange) and some with iron-hæmatoxylin.

The early stages of the spirem have not yet been studied, but the spirem forms a broad and probably continuous band. Before it segments, it often shows a differentiation, where not overstained, into a ground substance giving a pale purple or pale violet reaction with the gentian violet, and more deeply stained bodies which are in pairs and appear at quite regular intervals in the band. The line of separation which runs along between the pairs of denser chromatin masses marks the line of longitudinal division of the spirem, though the ground substance often shows no division line. As the spirem matures, here and there are seen short openings along the middle line. This is especially well marked as the spirem segments into the chromosomes. The individual chromosomes often show traces of longitudinal division, by short openings near one end, or at the middle, oftener at the ends, where there is a slight indentation; or, the division proceeding deeper, the end is more or less forked, resulting in Y- or U-shaped figures.

The spirem segments into about six chromosomes. They are at first rather long and become somewhat shorter and broader as they move to the nuclear plate of the spindle.

At this time the usual changes in the nucleus take place. The membrane disappears and the threads of kinoplasm move in, showing first a radiating arrangement and gradually moving to converge into the two poles as the spindle is formed; the chromosomes are drawn toward the center and are finally oriented in the nuclear plate. The chromosomes are broad, flattened,

and irregularly oblong. There is considerable variation in size.

They are very characteristic in form and structure. A few show narrow slit-like openings in the middle. A few of this form become very short and the opening then is somewhat rounded, so that a ring form is the result. This is comparatively rare. Others are divided at the ends, some showing a slight emargination, while some are more or less deeply forked, showing a tendency to form X and Y figures. Combinations of these two types are also found, so that there will be an opening in the middle, while the ends are somewhat forked, or one end is deeply divided. More characteristic, however, is the tendency to a differentiation in the density of the chromatin, which is especially marked when the chromatin is not stained too deeply. This differentiation of the chromatin is of the same kind as that manifested in the spirem and shows the paired condition of the chromosomes. The ground substance of the chromosome in these cases shows uniformly a paler tint and is translucent, while the denser masses of chromatin stain very dark and are more or less opaque. These chromatin masses are paired just as they are in the spirem, and probably result from the division of single chromatin masses in the early fission of the spirem. When these chromatin masses are well marked in the chromosomes, they seem to be uniformly of the same number so far as observed. There are often found well marked pairs of chromatin masses, each mass lying in the edge of the bar, four at the ends (two at each end) and four near the middle (two on each side near the middle), making eight in all.

Very frequently the edge of the bar is undulate, the prominences on the edges occurring at the location of the chromatin masses. The apparent uniformity in the number of these chromatin masses in the chromosomes is, perhaps, of considerable interest, and one is led to inquire whether they represent the units of the chromosomes or whether each one is a member of a tetrad group. If the latter is the true interpretation, then there would be in each chromosome of *Trillium grandiflorum* two united tetrads, or the chromosomes in the prophase of the

heterotypic division would be quadrivalent, instead of bivalent, as in the case of the normal tetrad.

As the chromosomes are drawn toward the nuclear plate, they become bent, so that each one represents a short arc of a circle or a broad open U form, as if drawn more forcibly by threads attached to the middle portion. As they are oriented on the nuclear plate transverse to the axis of the spindle they at first stand in various positions. Some lie tangentially, with the convex side toward the axis of the spindle and with both ends at the periphery, while others lie so that one end is directed toward the axis, with the other end at the periphery. In this way they are sometimes "convolute" or more or less "imbricate" in the nuclear plate, and may remain so for some time during the metaphase, or while separation at the nuclear plate is beginning to take place. Before separation has proceeded far, however, the chromosomes are usually more bent upon themselves, with the free ends of the U nearer together and directed outward in the usual way.

While the monaster is forming, and many of the chromosomes stand with one end directed toward the axis of the spindle and the other radiating therefrom, certain ones which are strongly lobed at the ends present a figure which leads one to think that the ends might be separated first, and that the chromosomes might then, in the anaphase, move to the poles in the form of a U, but with the concave or open side directed toward the poles, or that the inner ends might be separated first, and the chromosomes then move to the poles in the rod form. It does not seem, however, that the movement of the chromosomes has begun in such figures, and it is suggested that they are not yet drawn into proper position in the nuclear plate.

As separation of the paired chromosomes begins, the larger number of spindle threads appear to be attached in one tuft at the middle. The flattened U-shaped chromosome now broadens at the middle, in response to the tension of the tuft of threads attached at this point on either half, and the chromatin substance is soon drawn out into a short process on either side which

becomes the point of the V-shaped daughter chromosome, or forms a slight projection on the convex side of the U-shaped ones if this form is retained. No pull is exerted on the end of the chromosome, the ends of the pairs remain united, or in position at the periphery of the nuclear plate, while the middle portions are drawn toward the opposite poles, and in opening out thus, the dividing chromosome forms the diamond-shaped figure when seen from in front (*fig. 18*).

During the anaphase the chromosomes are V-shaped or U-shaped, and show considerable irregularity in contour, being more or less nodulose, often showing still the four dense chromatin masses. Some close up behind and form rings, some divide in front and form two rods. As the chromosomes approach the poles the polar ends converge, so that the chromosomes lie close together around the periphery of the ends of the spindle. Lying in this position the daughter nucleus is formed. The form of the daughter nucleus is somewhat like the half of a biconvex lens, the convexity being outward, while the truncated end lies toward the cell plate now formed by the connecting spindle threads. The form and position of the chromosomes give to the daughter nucleus its shape, for as the closed ends of the V- or U-shaped chromosomes converge at the apex of the cone, the spreading arms of the open end cause the nucleus to broaden out on the side facing the cell plate. The nuclear cavity now appears and the chromosomes are lying on its periphery against the nuclear membrane. They become usually more irregular in form, with angular points on the edge to which appear to be attached delicate threads connecting with the nuclear membrane, or reaching to an adjacent chromosome.

Where the nucleus is small by the close crowding of the large chromosomes, it is quite impossible to determine whether the chromosomes unite in such a way as to form a spirem. The nucleus does not, however, pass into a resting stage with the linin reticulum upon which the chromatin is distributed, but the chromatin bands remain intact. In the large nuclei, where the chromosomes do not lie so close together, they appear in most

cases to remain distinct, and in the form of V- or U-shaped bands, or often they are horseshoe-shaped, and sometimes form rings. In some cases it appears as if the arms of adjacent chromosomes coming in contact had fused at the end, but in no case could I see that they were thus united around the nucleus to form a continuous spirem, as described by Mottier for Podophyllum. Sometimes it appeared as if two had united by their ends to form a very large ring. In other cases the chromosomes may be permanently separated into two groups during this period.

As the nuclear cavity is formed and the first spindle is disappearing, the free ends of the chromosomes sometimes bend inward partly over the truncate side of the cavity, and at other times the chromosomes do not occupy the regular position which they usually show when they have reached the poles. In these cases ends of the chromosomes may be fused here and there, but in all stages several free ends are to be seen, and the figures presented by the nucleus are such as to lead one to believe that the chromosomes remain distinct through this phase. If the daughter nucleus is not elongated when first formed, it very soon begins to elongate in a plane parallel with the cell plates, so that it becomes nearly or quite twice as long as its diameter, and it is more or less inequilateral, the convex side being toward the periphery of the primary mother cell, while the plane side faces the cell plate. In opening out in this way the chromosomes become more and more distinct. The elongation of the daughter nucleus often takes place while the chromosomes are moving to the poles (*fig. 21*). In such cases the chromosomes are more easily followed, and the evidence is quite convincing in support of the view that the individuality of the chromosomes is preserved from the anaphase of the first division through to the prophase of the second mitosis. From the V- and U-shaped forms possessed by the chromosomes as they go to the poles, many of them change to horseshoe form, or some to complete rings by the free ends converging while the arms part slightly at the middle portion. In a number of cases the chromosomes divide

transversely at the convex end and the two rods lie side by side or near each other. The nuclear membrane now disappears and the kinoplasmic threads enter to form the spindle for the second division.

The elongation of the nucleus while the chromosomes are moving to the poles shows that the forces are in play which form the spindle for the second division. It is evident also that the chromosomes remain distinct, and that they are soon to be again separated transversely.

The elongated form of the nucleus marks the position of the spindle for the second division, and the poles of the spindle lie near the poles of the elongated nucleus. The spindle is therefore inequilateral, the poles being curved toward the cell plate of the first spindle figure. At the same time the chromosomes begin to move inward to the cell plate, and in doing so show the same general form which they possessed during the anaphase of the heterotypic division, which it seems they possess through the short period which intervenes before the formation of the second spindle figure.

During this time there is no longitudinal cleavage of the chromosomes. Even should the chromosomes form a continuous band in the daughter nucleus of the first division, there is no longitudinal splitting of the same at this time. Sometimes there is an appearance of a longitudinal fission of the chromosomes where the edges seem to be more deeply stained than a middle zone along the axis. This was found to be due, however, to the deeper staining of an outer layer of the large chromosome, and in properly stained preparations can be seen at any stage of mitosis.

If longitudinal fission of the chromosomes takes place during the second division, it would then be sought for in the nuclear plate stage.

Since the chromosomes during the prophase of the second mitosis are of the same general form as those of the anaphase of the first division, and very likely preserve their identity through the short intervening period, they should be chiefly V- or U-shaped. This is the case, as the examination of a large number

of preparations proves, while a few are ring form (*fig. 30*), and still others, having divided transversely at the apex of the V or the closed end of the U, exist as a pair of rods.

As the chromosomes approach the nuclear plate the arms of the V- or U-shaped ones close together, and, usually at the same time, transverse division takes place at the closed end. In this way all the chromosomes become of nearly uniform shape, consisting of two parallel or nearly parallel rods, which become more or less fused along the line of contact. The result of this is to form a double chromosome, very broad and irregularly oblong, which resembles in a striking manner the paired chromosomes at the nuclear plate of the first or heterotypic division, though they are formed as a result of folding and transverse division instead of by longitudinal cleavage. As the paired chromosomes are oriented on the nuclear plate, they usually become bent in such a way that the convex side lies toward the axis of the spindle, while the ends are directed outward, and the axis of the double chromosome is perpendicular to the axis of the spindle. The figure therefore presented by the metaphase of the second division is very much like that of the heterotypic division, and the only way in which one can determine that these belong to the second division is by the fact that there are two such spindles within the wall of the mother cell. Variations in their position in the monaster occur. One end of the chromosome may be directed inward toward the axis of the spindle, so that a few of them may be somewhat convolute; or the bent end may lie tangentially at the periphery of the spindle; or the chromosome may be nearly straight and standing on one end, while the other end radiates outward. As the position of the chromosome varies at the nuclear plate, so the figures presented during the anaphase vary, but the result is the same in each case.

The broad chromosomes lying thus at the nuclear plate, their edges face the poles of the spindle; the threads of the spindle which pull on the chromosomes are attached to that portion of the chromosome on either side which lies near the periphery of the spindle. On those which are bent so that both arms of the

U radiate equally, the threads are attached at the middle; on those which stand so that the arms radiate unequally the threads are attached somewhere between the end and the middle; while those which stand on the end have the threads attached at the end of each edge. The pull of the threads attached at the different points on the differently oriented pairs of chromosomes separate the individuals of the pair, the separation beginning at the point of attachment of the thread, and, as the portions are drawn toward the poles, the liberation proceeds until the members of the pair are no longer in contact. The different figures presented may be grouped all in three types, the V or U, the hook or the V with unequal arms, and the straight rods. These as can be readily seen are dependent on the point of attachment of the spindle threads.

The result of this separation of the individuals of the paired chromosomes in the second mitosis is a reducing division of the chromosomes, or a qualitative reduction of the chromatin substance; for, as we have seen, the paired chromosomes in the second mitosis are formed by the looping of longer chromosomes, which often open out at the bent end before reaching the nuclear plate. The second division results simply in the separation of the arms of this loop, and the distribution of each to a different daughter nucleus. It may be admitted here, then, with a feeling of reasonable confidence, that in *Trillium grandiflorum* a reducing division of the chromosomes, or in other words, a qualitative reduction of the chromatin takes place during the second division of the nucleus in the development of the pollen or microspores. One may well be cautioned against a hasty judgment in the interpretation of the figures presented during sporogenesis, because of what seems to be contradictory evidence given by different investigators upon the question as to whether or not a reducing division of the chromosomes takes place in plants, and it is only after careful study of an excellent series of preparations that I am led to present this as my conviction of the nature of the process as it occurs in Trillium. The large size and small number of the chromosomes, as well as

their form, have contributed in no small degree to the results obtained.

As is well known, the investigations of Mottier[4] upon division of the pollen mother cells in Podophyllum, led Strasburger[5] to believe that a reducing division of the chromosomes occurred during the second mitosis of the pollen mother cell. But more recent researches by Mottier, especially upon the condition of the chromosomes in the daughter nucleus and the origin of the chromosomes for the second division, led Strasburger[6] to recede from his former position and to reaffirm his conviction that the second division is the result of a longitudinal cleavage. According to Mottier, the chromosomes unite in the daughter nucleus of the first division in such a way as to form a continuous single band, *i. e.*, a band made of single chromosomes united end to end, the arms of the V-shaped chromosomes and of the paired ones, where transverse division has taken place, open out to join with the arms of neighboring ones. This band forms the spirem, which splits longitudinally before it segments into the chromosomes for the second division. As a result of this process of longitudinal fission of the spirem in Podophyllum, the segments or chromosomes are paired, but the pairs in the case of Podophyllum would then be the result of longitudinal fission, The separation of the members of the pair at the nuclear plate during the second mitosis would not then be here a reducing division.

These conclusions of Strasburger and Mottier led me to study very carefully the same stage in Trillium. As I have indicated above, the evidence seems to me to show that the chromosomes retain their individuality through the daughter nuclei from the anaphase of the first division to the prophase of

[4] MOTTIER, D. M.—Beiträge zur Kenntniss der Kerntheilung in den Pollen-mutterzellen einiger Dikotylen und Monokotylen. Jahrb. f. wiss. Bot. **30**: 169–204. *pl. 3–5*. 1897.

[5] STRASBURGER, E.—Ueber Cytoplasmastructuren, Kern- und Zelltheilung. Jahrb. f. wiss. Bot. **30**: 375–405. 1897.

[6] STRASBURGER, E. and MOTTIER, D. M.—Ueber den zweiten Theilungsschritt im Pollenmutterzellen. Ber. d. deutsch. bot. Gesells. **15**: 327–332. *pl. 15*. 1897.

the second. It might, however, be admitted that they unite to form a continuous spirem, without invalidating the conclusions reached in this study, for I have not found the slightest evidence of a longitudinal fission during the daughter nucleus stage. On the other hand the chromosomes, as the nucleus opens out, appear in the same form and number as shown at the close of the first division, that is, in the form of a letter V or U, or in rings, or even in the form of paired rods, which form more or less the same figure, and agree in this respect with the same types described by Beliaieff for the same stages.

The origin and form of the chromosomes in the first division of the pollen mother cell of Trillium, and the figures presented at the nuclear plate, show that the first division is heterotypic, though the transverse division of the chromosomes at this time, which indicates the tetrad character, is rarely present. The figures presented by the second division are exceedingly interesting, since they suggest the heterotypic division also, as is indicated by rings, in some cases, which are formed by the closing of the open ends of a V or U figure. There is thus a semblance of a heterotypic figure, with reducing or transverse division during the second mitosis in the sporogenesis of Trillium, and it would be interesting to know if the heterotypic figure described by Farmer[7] during the second mitosis in the sporogenesis of some liverworts, results in qualitative reduction. The figures presented by the chromosomes at the nuclear plate of the second division are strikingly similar to those which exist during the true heterotypic mitosis, and the separation of the chromosomes gives, during the anaphase, figures exactly like those shown during the first division.

A larger number of chromosomes in the second division, perhaps, are of the hooked form, and some are of the rod form, though both the hook and rod form are found rarely in the first division. It would appear that in Trillium, as well as in Arisæma, as shown by my studies on reducing division in that genus, the

[7] FARMER, J. B.—On spore formation and nuclear division in the Hepaticæ. Ann. Bot. 9:469–523. *pl. 16–18.* 1895.

form of the chromosomes and the mode of separation at the nuclear plate would not permit of classification into the types suggested by Beliaieff.[8] It is quite reasonable to believe that in mitosis, where the size and proportional dimensions of the chromosomes vary, as they are so well known to do, a great variety in the form of the chromosomes may exist, and that, correspondingly, there may be great variation in different species manifested in chromatin figures and evolutions, even where the same result is finally obtained.

In the peculiarities of the second mitosis of the pollen mother cell in *Trillium grandiflorum*, there is presented a distinct type in the evolutions of the chromosomes during the reducing division. Considerable interest attaches also to the peculiarity of the large chromosomes during the anaphase and metaphase of the first mitosis, where there is an appearance of eight denser portions of chromatin in the chromosomes, arranged in four pairs in such a way as to suggest two tetrad groups in a single segment of the spirem. In connection with this, it is interesting to note, that, while in *Trillium grandiflorum* during the first mitosis in the pollen mother cell there are only six chromosomes, in a number of the Liliaceæ there are twelve. This indicates, possibly, that the segments of the spirem in Trillium here represent four chromosomes, fused end to end (quadrivalent), instead of two (bivalent), which is usually the case as a result of the pseudo-reduction. *Arisæma triphyllum*, according to my studies on this species, also presents a distinct type, in that the reducing division, though following the longitudinal division, occurs during the first mitosis.

That there should be variations in the evolutions performed by the chromosomes in different plants, such as to represent different types, is what one might expect, not only in view of the great variations in the size and form of the chromosomes in different plants, representing different types of chromosomes, but also in view of the tendency to variation so manifest in many of

[8] BELIAIEFF, W.—Ueber die Reductionstheilung des Pflanzenkernes (Vorläufige Mittheilung). Ber. d. deutsch. bot. Gesells. **16**: 27–34. 1898.

the phenomena of plant life, extending even to nuclear phenomena, illustrated by the different types in fertilization, as shown by the investigations of Ikeno[9] in Cycas, Shaw[10] in Onoclea, and Blackman[11] in *Pinus sylvestris*, as well as by Miss Ferguson[12] in *Pinus Strobus*.

Is it not well to inquire if some of the divergent and contradictory results regarding the behavior of the chromosomes obtained by different investigators, when dealing with different plants, are not due to the fact that we are dealing with different types in some cases? Are there not among plants different types of chromatin reduction? So that in one type there is represented a mass reduction, or quantitative reduction of the chromatin; in another type a pseudo-reduction, or numerical reduction only of the chromosomes; and in another type a qualitative reduction of the chromatin or reducing division of the chromosomes? Touching the hereditary or constitutional influences of fertilization, we recognize different types in plants, as shown by close- and cross-fertilization; and different types also in the mechanism for bringing about pollination.

Some of the bewilderment which now surrounds certain phases of the study of the morphology of the nucleus will, I believe, disappear, if we recognize that there is such a thing as a reducing division or qualitative reduction in plants as represented by such types as Trillium, Arisæma, Adiantum, Pteris, Iris, etc.; that there are plants in which only a quantitative or numerical reduction occurs, represented by such a type as Podophyllum; and possibly that there is still another type, where

[9] IKENO, S.—Untersuchungen über die Entwickelung der Geschlechtsorgane und den Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. f. wiss. Bot. **32**: 557–602. *pl. 8–10*. 1898.

[10] SHAW, W. R.—The Fertilization of Onoclea. Ann. Bot. **12**: 261–285. *pl. 19*. 1898.

[11] BLACKMAN, V. H.—On the cytological features of fertilization and related phenomena in *Pinus sylvestris* L. Phil. Trans. Roy. Soc. B. **190**: 395–426. *pl. 12–14*. 1898.

[12] Miss Ferguson's studies were carried on under my direction in the Bot. Lab. Cornell University, and a paper, yet unpublished, was read before the Bot. Soc. Am. Aug. 1898, entitled "A preliminary note on fertilization in the white pine."

in the same plant qualitative reduction may take place in some cells, while quantitative or numerical reduction only takes place in others. This seems to me, as a working hypothesis, more reasonable than to insist, because one type has been found in one or several plants, that all plants must conform to it.

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EXPLANATION OF PLATES I–VI.

(All the figures are drawn to the same scale, using a Zeiss microscope, compensation ocular 12, and the 2^{mm} homogeneous immersion objective, the image being thrown down even with the base of the microscope. The figures are reproduced from these drawings without any reduction.)

PLATES I AND II. Arisæma triphyllum.

FIG. 1. Spirem stage of nucleus showing the dividing thread with chromatin masses on it. The masses of chromatin are partly divided, and appear as paired masses along the thread.

FIG. 2. Portions of partly formed spirem, showing indications of longitudinal division.

FIG. 3. Early stage of chromosomes, just after segmentation of spirem band, showing different stages of longitudinal division, forming oblong plates with ends slightly forked, or those approaching X and U forms, where the division is more marked. Fragments of linin are still attached.

FIGS. 4, 5, 6, 7. Different stages, showing the gradual shortening of the chromosomes in some cases, in others different forms of chromosomes, in same stage as those in *fig. 3*. In *figs. 4* and *5*, some are opening out to form rings; in *fig. 6* one of the oblong rings is twisted to form a figure 8, and in *fig. 7* one shows two openings along the line of longitudinal division. All of the chromosomes in these figures are yet more or less irregular, angular, and show fragments of the linin attached.

FIGS. 8, 9, 10. Chromosomes much shortened, and a large number of them in the ring form. In *fig. 8* two have completely divided to form rods, two form oblong plates with forked ends, and one forms a ring, while in *fig. 10* nearly all are in the ring form. Some of them are still angular, and show the peculiar form of so many of the rings of Arisæma. The tetrads are being formed by the accumulation of the chromatin in denser portions near the ends of the rods, and near the ends of each half of the rings, the paler zone across the middle showing the line of transverse division. Linin threads are still attached to some of the angles. The nuclear membrane is still intact, and the chromosomes are arranged around the periphery of the nuclear cavity in the positions occupied by them when they separated as segments from the spirem.

FIGS. 11, 12, 13. The nuclear membrane has disappeared, and threads of kinoplasm are entering to form the spindle; chromosomes in the form of rings, paired rods, or angular plates, which are solid or with a central perforation indicating the ring form.

FIG. 14. Spindle with chromosomes approaching the nuclear plate. The poles of the spindle show radiating lines of protoplasm indicating centers of force.

FIG. 15. Chromosomes in the nuclear plate stage, but they are in this preparation so fused that it is impossible to see the way in which division takes place.

FIGS. 16, 17. Chromosomes beginning to separate at the nuclear plate; rings, paired rods, and tetrads can be seen. The rings and paired rods are lying with the long axis parallel with the axis of the spindle, longitudinal division has taken place in some and is taking place in others, so that the halves of rings form crescent-shaped rods, the tetrads are indicated by deeper staining portions of the rods or rings.

FIGS. 18, 19. A little later condition of the same (metaphase) stage; longitudinal division has taken place so that nearly all the rods or halves of the rings have separated, and by the pulling of the spindle threads the tetrads are being drawn apart transversely to the axis of the chromosome, to bring about the reducing division.

FIGS. 20, 21. Polar view of the metaphase, showing that the chromosomes are distributed all through the nuclear plate. Sixteen groups can be counted, bearing in mind that a pair of rods, or a tetrad, makes a group. Some of the chromosomes are turned somewhat obliquely, especially in *fig. 20*, which was an oblique view of the plate, and in some cases the paired rods are seen only from the end.

FIG. 22. The tetrads have nearly separated by transverse division.

FIGS. 23, 24. Tetrads, about 32 on each side, approaching the poles during the anaphase of the first mitosis.

PLATES III–VI. Trillium grandiflorum.

FIG. 1. Portion of the spirem with partial longitudinal division, and a few chromosomes.

FIGS. 2, 3, 4, 5. Early stage in the formation of the chromosomes just after the segmentation of the spirem, showing, in some, openings along the middle line, in others the ends forked, indicating the line of longitudinal division. Pairs of deeply staining chromatin masses are evident in some of the chromosomes, frequently four pairs, or eight masses in a single chromosome.

FIGS. 6, 7, 8, 9, 10. The nuclear membrane has disappeared, and the chromosomes are being drawn up to the nuclear plate.

FIGS. 11–17. Various figures showing the metaphase stage, the orientation of the chromosomes in the nuclear plate; in some, as in *fig. 11* and *17*, the four paired masses of denser chromatin are well seen; in *fig. 17* the pull of the spindle threads is drawing out the middle portion on either side of the broad chromosome, the first step in the separation of the longitudinally divided segment.

FIGS. 18, 19. Two different stages in the separation of the chromosomes in the V form as they are pulled toward the poles.

FIG. 20. Cell plate formed after first mitosis, and U or horseshoe-shaped chromosomes lying in position as they approached the poles.

FIGS. 21, 22. Oblique view of two poles, to show the position of the chromosomes as they come to the poles; in *fig. 21* the group has already elongated somewhat in the direction of the axis of the spindle for the second mitosis.

FIGS. 23, 24, 25, 26. Position of the chromosomes in the daughter nucleus after first mitosis; nuclear membrane formed.

FIG. 27. Nuclear membrane has disappeared at the prophase of the second mitosis, and the chromosomes, still showing the form and characters present when reaching the poles after the first mitosis, are beginning to move to the nuclear plate of the second mitosis.

FIGS. 28, 29, 30. Later stages as the chromosomes are approaching the nuclear plate. The U form is closing up by the folding of the arms, and in some, transverse division has taken place so that they form a pair of rods; in a few the ends of the arms have met and form a ring, which simulates the form of chromosomes in the heterotypic division.

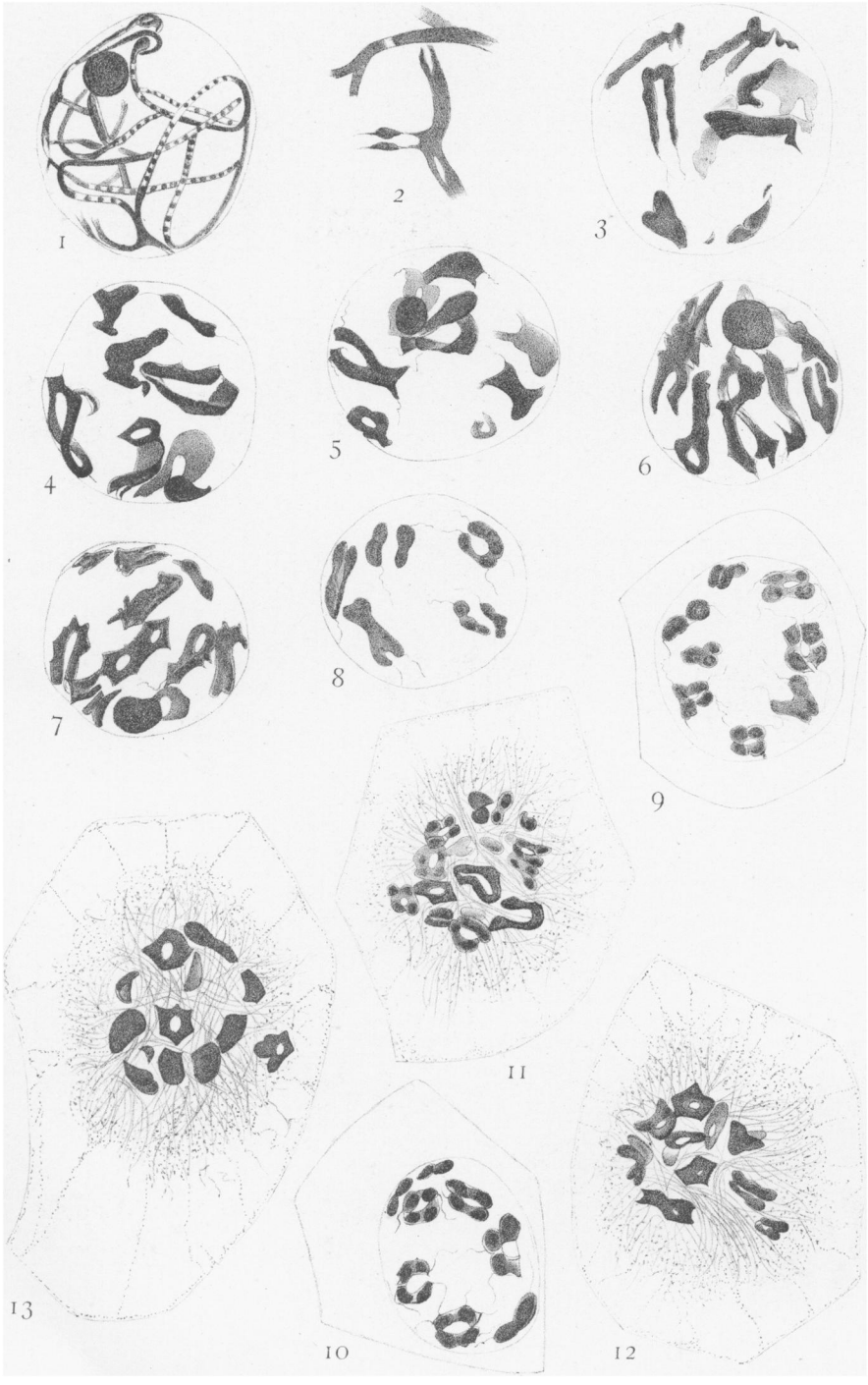
FIG. 31. End view of nuclear plate stage in second mitosis.

FIG. 32. Side view with spindle established, the chromosomes presenting much the same form as in the first mitosis, from the closing up of the arms of the U and the cross division at the junction of the arms.

FIGS. 33, 34, 35. Different stages in the separation of the transversely divided chromosomes at the nuclear plate in second mitosis.

FIG. 36. Three different forms of the chromosomes as they are passing to the poles in the second mitosis; the V form; hooked, or J form; and the rod form, depending on the point of attachment of the spindle threads; all in the same nucleus.

FIGS. 38, 39. End view of spindle showing chromosomes approaching the poles at the close of the second mitosis.



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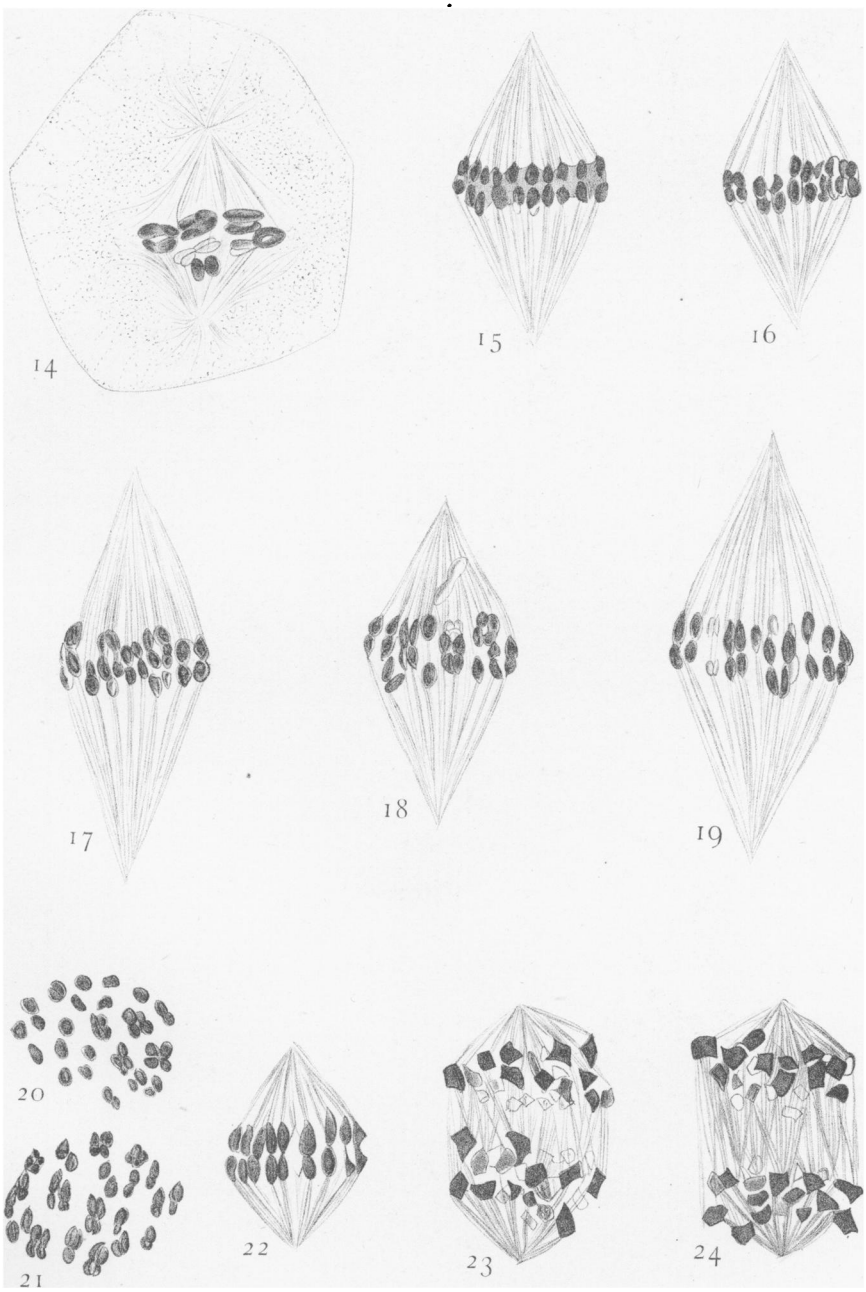
ATKINSON on REDUCTION of CHROMOSOMES



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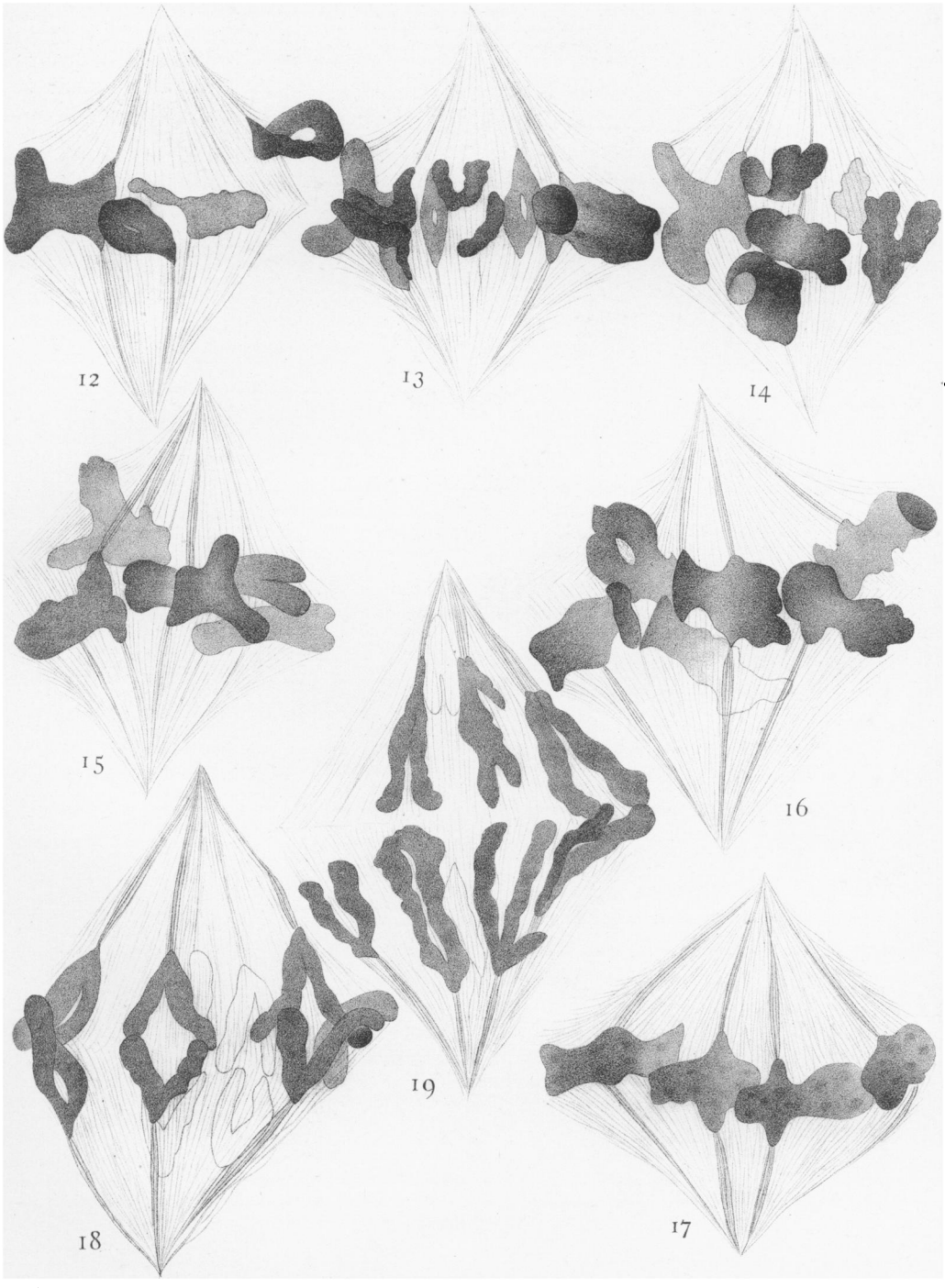

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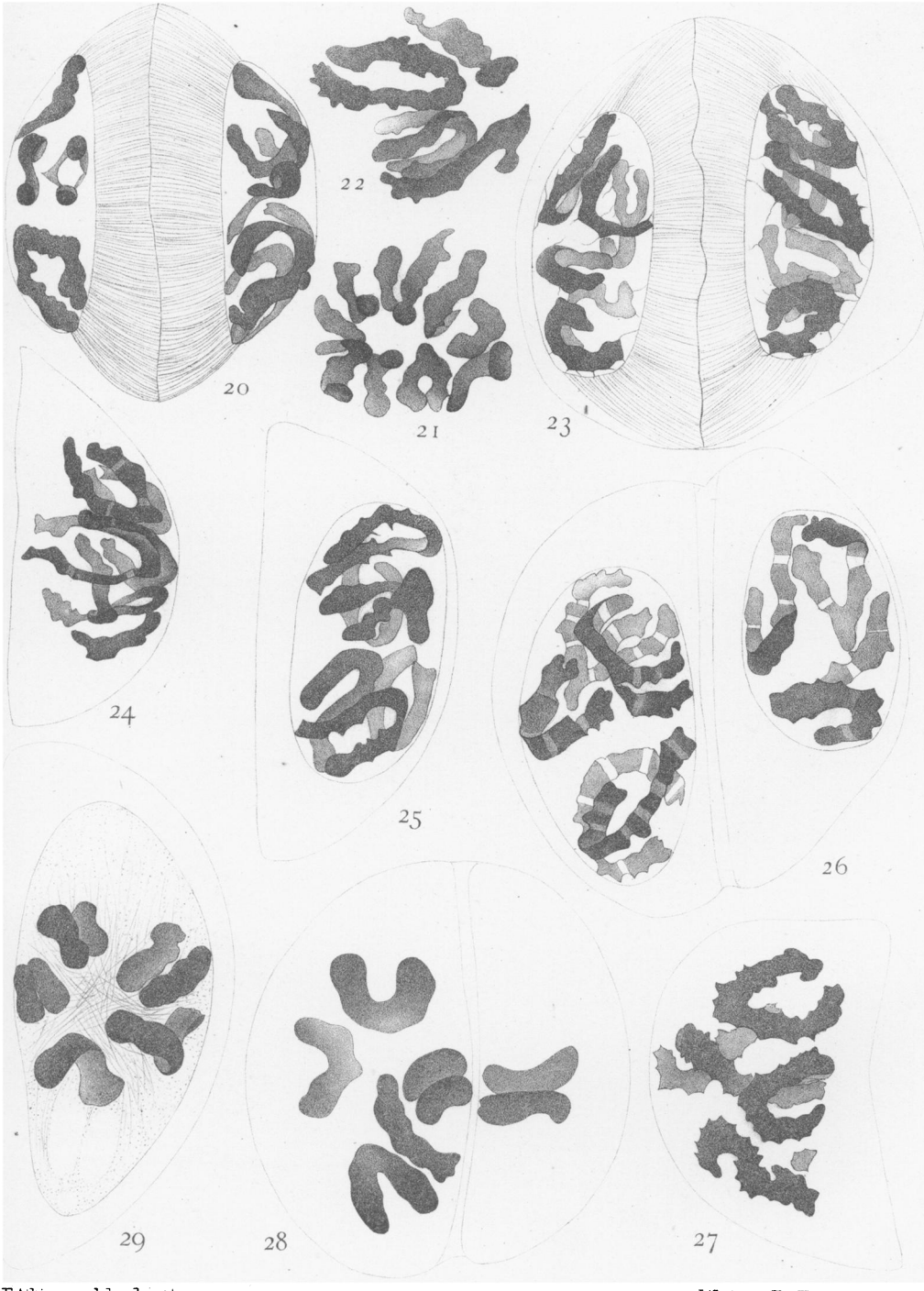

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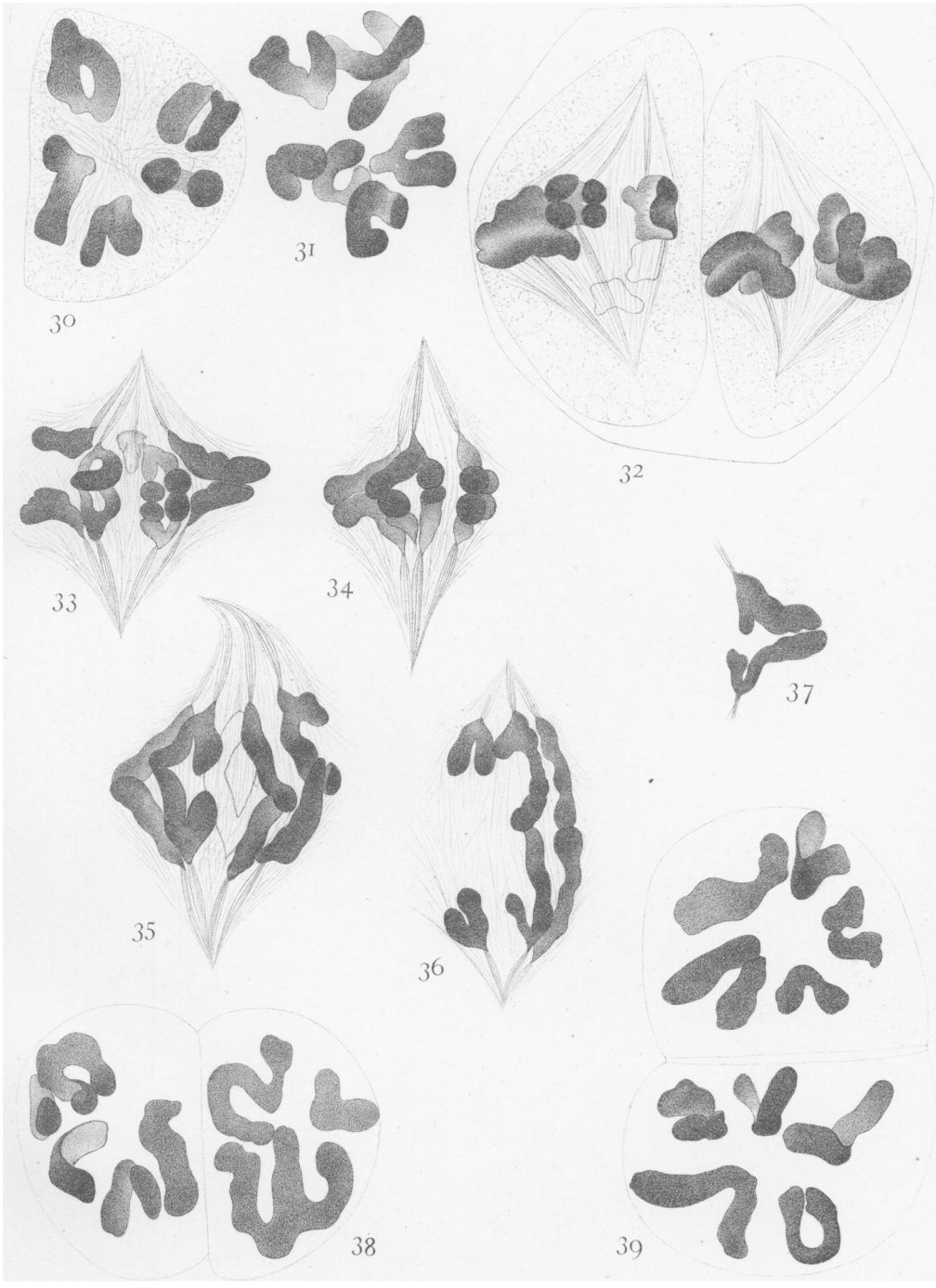

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